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Statistical Analysis Plan

Study Title: A Presurgical Tissue-Acquisition Study to Evaluate Molecular Alterations in Human Breast Cancer Tissue Following Short-Term Exposure to the Androgen Receptor Antagonist Darolutamide (ODM-201)

Study Number: TRIO030

Version, Date: 3.0, 30 September 2019

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Revision History

The subjective version of the SAP 3.0 is updated to align with the latest protocol revision, and update the sample size as a result of the enrollment closure dated 14 February 2019. Briefly, only seven patients in the triple negative subgroup have been enrolled; one less than planned as per protocol.

Table 1 – Revision History

Section #	Type of Change	Change
3.5.1	Content	Removed formula 'c' for Duration of Treatment (days) and Treatment Exposure (days)
4.1	Content	Added additional description for Laboratory Values and ECOG Performance Status to include total number of patients

Table of Content

List of Abbreviations

ADSL	Subject-Level Analysis Dataset
ADaM	Analysis Data Model
AE	Adverse Events
CM	Concomitant Medications
CTCAE	Common Terminology Criteria for Adverse Events
EBC	Early Breast Cancer
EoS	End of Study
ITT	Intent-to-treat or Intention-to-treat
ITTFL	Intent-to-treat Population Flag
MedDRA	Medical Dictionary for Regulatory Activities
NCI	National Cancer Institute
PT	Preferred Term
Q1	First Quartile
Q3	Third Quartile
RDI	Relative Dose Intensity
SAE	Serious Adverse Event
SAFFL	Safety Population Flag
SAP	Statistical Analysis Plan
SAS®	Statistical Analysis System Software
SOC	System Organ Class
SSC	Study Steering Committee
TEAE	Treatment Emergent Adverse Event
TRIO	Translational Research in Oncology
WHODD	World Health Organization Drug Dictionary

1. Introduction

This Statistical Analysis Plan (SAP) describes the detailed statistical methodology for executing the statistical analyses of the Secondary Objective according to TRIO030 protocol version 3.0 and version 5.0 (Germany).

Statistical analysis will be performed by TRIO using SAS® software Version 9.4 or higher.

2. Study Design and Objectives

2.1. Study Design

The protocol synopsis is provided in Appendix 5.1 and Appendix 5.2. Refer to the protocol for additional details.

2.2. Study Objectives

2.2.1. Primary Objective

The primary objective for this study is to identify the molecular alterations that occur in human Breast Cancer (BC) tissue, following short-term exposure to darolutamide (ODM-201) in female subjects with Early Breast Cancer (EBC).

2.2.2. Secondary Objective

To evaluate safety and tolerability following short-term exposure to darolutamide (ODM-201) in female patients with EBC.

2.3. Sample Size

Up to 60 patients (a minimum of eight evaluable patients, and up to 20 patients in each BC Subtype: triple-negative, HR+/HER2 negative, HER2 positive) will be required for the performance of the molecular trials and possible hypothesis generation.

At the time of enrollment closure on 14 February 2019, 36 patients were enrolled with the following characteristics per local or central assessment: seven triple-negative, 19 HR+/HER2 negative, and 10 HER2 positive. Although the triple-negative subgroup did not reach the minimum number of eight planned patients as per protocol, it was deemed acceptable for the following reasons:

- The primary objective of the study is to identify the molecular alterations that occur in human BC tissue, following short-term exposure to darolutamide in female patients with EBC. There are no efficacy objectives in this trial.
- The number of patients per cohort was not defined after a formal sample size calculation, but was a number considered appropriate for the performance of the molecular studies according to the recommendations by the central laboratory responsible for the molecular analyses.
- The central laboratory now considers that seven patients in the triple-negative cohort is sufficient to perform the evaluation of the molecular alterations, and to eventually

generate the relevant hypothesis in this subgroup. Seven patients is then deemed sufficient to adequately characterize the study objectives in this tissue-acquisition window of opportunity study.

- The Sponsor, along with the Study Chair, have determined that trial objectives have been reached in terms of the sample banking needed for biomarker analyses.
- In terms of statistical analysis (specifically the safety profile), there is no comparison between subgroups, so this change will have no effect to the final analysis.

2.4. Timing of Analyses

Safety outputs will be generated for data review performed by the Study Steering Committee (SSC) once 20, around 35, and once all patients have been enrolled and undergone the End of Study (EoS) as per protocol.

A Final Safety Analysis will be performed when all patients have completed the trial.

3. Statistical Methods

3.1. Analysis Population

3.1.1. Intention-to-Treat Population

The **Intention-to-Treat (ITT) Population** includes all patients who are enrolled in the trial, regardless of whether they actually received investigational medication. This population will be identified using the Intent-to-Treat Population Flag (ITTFL) as defined in Subject-Level Analysis Dataset (ADSL) according to Analysis Data Model (ADaM).

3.1.2. Safety Population

The **Safety Population** includes all patients who receive at least one tablet of darolutamide, and will be used for the analysis of safety data of the trial. This population set will be identified using the Safety Population Flag (SAFFL) as defined in ADSL according to ADaM.

3.1.3. Evaluable Population

The **Evaluable Population** includes all patients who satisfied the following criteria:

- Minimum duration of treatment with darolutamide is 14 days, with a minimum of 10 consecutive days of treatment prior to the definitive surgery.
- Mandatory tumor tissue is collected at screening and at surgery according to the protocol and submitted to the central laboratory.
- Adequacy for molecular assessment of the tumor tissue collected before and after the protocol treatment initiation. The adequacy will be evaluated by the central laboratory.

The evaluable population will be used for the performance of molecular trials and possible hypothesis generation. More information regarding Molecular Analyses will be discussed in a separate document.

3.2. Data Handling

3.2.1. Baseline Assessment/Measurement

The last available assessment/measurement of a particular data point on or prior to the latter of the date of enrollment and the date of first dose of investigational treatment. If the patient has no value as defined above, the baseline value will be missing.

3.2.2. Date of First Dose

The date of first dose (of investigational drug) is derived as the first date when a non-zero and non-missing dose of investigational drug is administered. This is also referred to as the start of the treatment.

3.2.3. Date of Last Dose

The date of last dose (of investigational drug) is derived as the last date when a non-zero and non-missing dose of investigational drug is administered.

3.2.4. Duration

Duration, except for duration of treatment, is calculated as:

- Duration (days): (End Date – Start Date + 1).
- Duration (weeks): (End Date – Start Date + 1) / 7
- Duration (months): (End Date – Start Date + 1) / 30.4375
- Duration (years): (End Date – Start Date + 1) / 365.25

3.2.5. Missing Data

All analyses and descriptive summaries will be based on the observed data. Unless otherwise specified, missing data will not be imputed or “carried forward.”

3.2.6. Partial Dates

Only partial or missing dates for Adverse Events (AE) and Concomitant Medications (CM) will be imputed according to the following:

3.2.6.1. Imputation Rules for Partial or Missing Stop Dates:

If the month and year are present, impute the last day of that month.

If only the year is present, impute December 31 of that year.

If the stop date is entirely missing, assume the event or medication is ongoing.

If a partial or complete stop date is present and the ‘ongoing’ box is checked, then it will be assumed that the AE or CM stopped and the stop date will be imputed if partial.

3.2.6.2. Imputation Rules for Partial or Missing Start Dates

Start Date		Stop Date						
		Complete: *yyyymmdd*		Partial: *yyyymm*		Partial: *yyyy*		Missing
		< 1st dose	≥ 1st dose	< 1st dose *yyyymm*	≥ 1st dose *yyyymm*	< 1st dose *yyyy*	≥ 1st dose *yyyy*	
Partial: *yyyymm*	= 1st dose *yyyymm*	2	1	2	1	n/a	1	1
	≠ 1st dose *yyyymm*		2		2	2	2	2
Partial: *yyyy*	= 1st dose *yyyy*	3	1	3	1	n/a	1	1
	≠ 1st dose *yyyy*		3		3	3	3	3
Missing		4	1	4	1	4	1	1

- 1 = Impute the date of first dose
- 2 = Impute the first of the month
- 3 = Impute January 1 of the year
- 4 = Impute January 1 of the stop year

3.3. Statistical Analysis

Categorical variables will be summarized in frequency tables, with the counts and percentage of patients in each category. Percentages given in the summary tables will be rounded and thus may not always add up to exactly 100 percent. For continuous variables, summary statistics will include number of patients, mean, standard deviation, first quartile (Q1), median, third quartile (Q3), and minimum and maximum values (range).

EBC Subtype determined by the central assessment will be used for statistical analyses.

3.3.1. Patient Disposition

The patient disposition will be tabulated using the ITT. Frequency count and respective percentages of patients who were in each population (i.e. ITT, Safety, and Evaluable), and discontinued from treatment and/or trial will be summarized by EBC Subtype. The reason for discontinuation (from treatment and/or from trial participation where applicable) will be summarized considering the categories specified in the case report forms. Listings of disposition information and analysis population will be provided as well.

Protocol deviations, including eligibility deviations of the inclusion/exclusion criteria, will be summarized and listed for ITT, by EBC Subtype.

3.3.2. Demographics and Disease Characteristics

The following patient demographics and disease characteristics will be summarized and listed for the ITT and Safety Population by EBC Subtype:

Patient Demographics:

- Age, the integer part of [(Date of enrollment – Date of birth) / 365.25)]
  - Note: Since only the year of birth is collected (YYYY), we assume the full date to be 31-Dec-YYYY
- Race
- Ethnicity
- Menopausal Status
- Weight at Baseline
- Height at Baseline
- ECOG Status at Baseline

Disease Characteristics:

- Time from Initial Diagnosis to Enrollment
- Primary Tumor Location
- Staging at Initial Diagnosis
- Histopathological Type at Initial Diagnosis
- Histopathological Grade at Initial Diagnosis
- Local Estrogen Receptor Status
- Local Progesterone Receptor Status
- Local HER2 Status

Medical History:

The medical history is coded by the most current version of Medical Dictionary for Regulatory Activities (MedDRA), and will be summarized for ITT by System Organ Class (SOC) and Preferred Term (PT) by EBC Subtype.

3.4. Efficacy Analyses

Not applicable.

3.5. Safety Analyses

Safety analyses will be performed on the **safety population**.

3.5.1. Extent of Exposure

Duration of Treatment, Treatment Exposure, Cumulative Dose and Relative Dose Intensity (RDI) will be summarized by EBC Subtype for the safety population.

- Duration of Treatment (days) = sum of duration of each administration period
  - Duration of each administration period = [(End Date - Start Date) + 1]
- Treatment Exposure (days) = [(Date of last dose - date of first dose) + 1]

- Cumulative Dose (mg) = sum of [duration of each administration period × Actual Daily Dose]
- Actual Dose Intensity (mg/day) = (Cumulative Dose) ÷ (Treatment Exposure)
- Planned Dose Intensity (mg/day) = 1200 mg
- RDI (%) = (Actual Dose Intensity ÷ Planned Dose Intensity) × 100

The RDI will be additionally presented categorized (i.e., number and percentage of patients with RDI of < 60%, 60 - < 80%, 80 - < 90%, 90 - < 110%, ≥ 110%).

The number and percentage of patients with dose modifications, along with their respective reasons will be summarized.

The compliance is assessed for the entire treatment period and will be calculated as:

$$\text{Compliance (\%)} = \frac{\text{Number of Tabs Taken}}{\sum[\text{Duration of each administration period} \times (\text{Actual Daily Dose} \div 300)]} \times 100$$

3.5.2. Adverse Events (AEs)

AE information will be collected from the time the patient signs the informed consent and continues throughout the trial until EoS. After EoS, AE/SAE information will be collected only if related to the study treatment.

The reported AE term will be coded using the current version of the MedDRA. The severity of AE will be presented as reported by the Investigator based on National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) grades version 4.03.

Treatment-Emergent Adverse Events (TEAEs) is defined as AEs that start on or after the first dose of investigational treatment or AEs that start before the first dose of treatment but worsened after investigational treatment is initiated.

Incidence of TEAE will be tabulated by SOC, PT for:

- All TEAEs
- TEAEs by relationship to investigational treatment and maximum severity grade
- TEAEs with action taken dose reduced/drug interrupted
- TEAEs with action taken drug withdrawn
- Serious Adverse Events (SAE) (not Serious TEAEs)
- AEs leading to deaths (not Fatal TEAEs) – An AE is considered as leading to death if it reported as Grade 5.

In the event a patient experiences repeat episodes of the same AE according to PT, the event with the highest severity grade will be used for purposes of incidence tabulations.

Detailed listings for all AEs will be provided, including listing with AEs per cohort. A flag will be used to identify which AE is considered as treatment emergent.

3.5.3. Laboratory Values

Lab values will be converted to standard units and categorized according to NCI-CTCAE version 4.03. Laboratory results not corresponding to NCI-CTCAE terms will not be graded.

Hematology and serum blood chemistry will be summarized in descriptive statistics by calculating the mean, standard deviation, median and range for the following:

- Baseline value
- Minimum post baseline value
- Maximum post baseline value

Shift tables in laboratory toxicity grades (comparing baseline grade with worst post-baseline grade) will be analyzed using standard shift tables, presenting number and proportion of patients and their maximum grade shift.

3.5.4. ECOG Performance Status

Tabulation of ECOG Performance Status results will be done for the following:

- Baseline value
- Minimum post baseline value
- Maximum post baseline value

A shift table will be provided as well from baseline status to the worst status of post-baseline time points.

3.5.5. Concomitant Medications

Prior and concomitant medications are coded to the current version of World Health Organization Drug Dictionary (WHODD). The Preferred Name will be tabulated and listed by patient.

3.5.6. Death

All deaths will be reported in a patient listing, which will include date of death, date of last dose, the primary cause of death, and the number of days between the date of last dose of investigational drug and death (if applicable).

4. List of Planned Statistical Outputs

4.1. Planned Tables

Title	Population	Description
Patient Disposition	ITT	Tabulates the disposition of all patients by EBC Subtype, including the number of patients in each population set as well as the number of patients discontinued from investigational treatment, and the number of patients discontinued from trial. The reason for drug discontinuation and trial discontinuation will also be summarized.
Protocol Deviations	ITT	Tabulate the number of patients who reported protocol deviations by deviation category for each

Title	Population	Description
		EBC Subtype.
Eligibility Deviations	ITT	Tabulates the number of patients who violates inclusion/exclusion criteria by criterion for each EBC Subtype.
Patient Demographics	ITT Safety	Tabulates summary statistics of the demographics (age, race, ethnicity, menopausal status, weight, height and ECOG status at baseline).
Disease Characteristics	ITT	Tabulates summary statistics for time from initial diagnosis to enrollment, primary tumor location, staging at initial diagnosis, histopathological type at initial diagnosis, histopathological grade at initial diagnosis, hormonal receptor status, and HER2 status.
Medical History	ITT	Summarizes medical history by SOC and PT for each EBC Subtype.
Study Drug Exposure	Safety	Summarizes the duration of treatment, cumulative dose, and RDI by EBC Subtype. The number of dose modifications will also be summarized.
Compliance	Safety	Compliance assessment will be summarized.
TEAEs Overview	Safety	Tabulates the number of patients with TEAEs, Related TEAEs, Grade 3 / 4 TEAEs, TEAEs with action taken as dose reduced or drug interrupted, AEs leading to drug withdrawn, SAE, Fatal AE.
TEAEs	Safety	Summary of number of patients and events by SOC and PT for each EBC Subtype.
TEAEs per Maximum Grade	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.
Related TEAEs	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.
Related TEAEs per Maximum Grade	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.
Grade 3 / 4 TEAEs	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.
TEAEs with Action Taken as Dose	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.

Title	Population	Description
Reduced		
TEAEs with Action Taken as Drug Interrupted	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.
TEAEs Leading to Treatment Discontinuation	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.
SAE	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.
AEs Leading to Death	Safety	Summary by SOC and PT of number of patients and events for each EBC Subtype.
Laboratory Values	Safety	Summary of each laboratory parameter at baseline, minimum post baseline value, and maximum post baseline value for each EBC Subtype and the total number of patients. In addition, shift tables will be presented.
ECOG Performance Status	Safety	Summary of each laboratory parameter at baseline, minimum post baseline value, and maximum post baseline value for each EBC Subtype and the total number of patients. In addition, shift tables will be presented.
Concomitant Medications	Safety	Tabulate the frequency and percentage of patients according to Preferred Name. Summary will be presented separately for prior medications and concomitant medications.

4.2. Planned Listings

Title	Population	Description
Patient Accountability	ITT	Listing of all patients and with population indicators, EBC Subtype. For patients who discontinued from investigational treatment and/or trial, include the reason of respective discontinuations.
Demographics	ITT	Listing of demographics.
Disease Characteristics	ITT	Listing of disease characteristics.
Local Hormonal	ITT	Listing of local hormonal receptors and HER2

Title	Population	Description
Receptors and HER2 Status		status.
Deaths	ITT	Listing of patients who died, including primary cause of death, date of death, date of last dose, and the number of days from date of last dose to death (if applicable).
Study Drug Exposure	Safety	Listing of patients for extent of exposure.
Study Drug Exposure Summary	Safety	Listing of patients for summary of exposure (e.g. duration of treatment, cumulative dose, RDI).
AEs	Safety	Listing of all AE for each patient; the listing includes the verbatim term, PT, SOC, seriousness, worst grade, start date, outcome, stop date, relationship to study drug, action taken for study drug, and treatment emergent flag.
Related TEAEs	Safety	Listing of TEAEs in this category using the same structure as the AEs listing with the omission of treatment emergent flag.
Grade 3 or 4 TEAE	Safety	Listing of TEAEs in this category using the same structure as the AEs listing with the omission of treatment emergent flag.
TEAEs with Action Taken for Dose Reduced	Safety	Listing of TEAEs in this category using the same structure as the AEs listing with the omission of treatment emergent flag.
TEAEs with Action Taken for Drug Interrupted	Safety	Listing of TEAEs in this category using the same structure as the AEs listing with the omission of treatment emergent flag.
TEAEs Leading to Study Treatment Discontinuation	Safety	Listing of TEAEs in this category using the same structure as the AEs listing
SAEs	Safety	Listing of AEs in this category using the same structure as the AEs listing.
Related SAEs	Safety	Listing of AEs in this category using the same structure as the AEs listing.
AEs Leading to Death	Safety	Listing of AEs in this category using the same structure as the AEs listing.
Laboratory Values	Safety	Listing of all laboratory values and their assigned

Title	Population	Description
		toxicity grade (where applicable).
ECOG Performance Status	Safety	Listing of ECOG for each reported assessment.
Prior and Concomitant Medications	Safety	Listing of all prior and concomitant medications; the listing includes verbatim product name, generic name, start date, end date (or ongoing) and indication.

4.3. Planned Graphs

Not applicable.

5. Appendix

5.1. Protocol Synopsis – Protocol v3.0

Protocol Title	A Presurgical Tissue-Acquisition Study to Evaluate Molecular Alterations in Human Breast Cancer Tissue Following Short-Term Exposure to the Androgen Receptor Antagonist Darolutamide (ODM-201)
Protocol #	TRIO030
Study Duration	Enrollment is expected to run over a 12-month period approximately. The overall study is expected to last around 14 months (from First Patient In until Last Patient Last Visit).
Sponsor/Study Chair	This is a TRIO-sponsored study which will be chaired by Dr. Dennis Slamon (Professor of Medicine, Chief, Division of Hematology-Oncology, UCLA's Department of Medicine, CA, USA). The study is financially supported by Bayer.
Participating Investigators/Sites	Approximately 20 sites in North America and Europe will participate in the study.
Target Population	Women with early-stage breast cancer (BC) candidates for surgery as primary treatment modality or candidates for neoadjuvant treatment who accept to undergo a second biopsy before starting such therapy
Background and Rationale	Despite progresses made in diagnostic techniques and treatments for BC, there is still a significant number of patients undergoing treatment for early BC (EBC) who will relapse or patients with advanced BC that do not respond or progress to their treatments. The medical need for new and potentially more active and better tolerated agents in BC remains very high. Endocrine treatment is one of the strategies with a major therapeutic value in patients with estrogen-receptor (ER) positive tumors. Although, the role of ER and progesterone receptor (PgR) is well established in BC as predictive and Despite progresses made in diagnostic techniques and treatments for BC, there is still a significant number of patients undergoing treatment for early BC (EBC) who will relapse or patients with advanced BC that do not respond or progress to their treatments. The medical need for new and potentially more active and better tolerated agents in BC remains very high. Endocrine treatment is one of the strategies with a major therapeutic value in patients with estrogen-receptor (ER) positive tumors. Although, the role of ER and progesterone receptor (PgR) is well established in BC as predictive and prognostic factors, little is known

	about the clinical significance of the androgen receptor (AR). Preclinical studies show that the androgen signaling pathway plays a critical role in the development of normal and malignant breast tissue. AR is expressed in approximately 80% and 60% of primary and metastatic BC, respectively. Its expression varies across the different subtypes, approximately 84-95% in ER+ tumors, 50-63% in ER-/HER2+ cases and 10-53% in TNBC. prognostic factors, little is known about the clinical significance of the androgen receptor (AR). Preclinical studies show that the androgen signaling pathway plays a critical role in the development of normal and malignant breast tissue. AR is expressed in approximately 80% and 60% of primary and metastatic BC, respectively. Its expression varies across the different subtypes, approximately 84-95% in ER+ tumors, 50-63% in ER-/HER2+ cases and 10-53% in TNBC. In ER+ BC it has been suggested that acquired resistance to antiestrogen therapies could result from adaptation from estrogen dependence to androgen dependence. Recently, clinical reports of AR antagonism have been promising, especially in TNBC. Bicalutamide and enzalutamide have been tested in phase II trials in TNBC and have shown promising clinical activity. These developments in combination with an improved understanding of AR signaling in different BC subtypes led to the renewed interest in targeting AR. However, the value of AR antagonists in the management of BC, the molecular alterations that occur after exposure to these agents and whether there are biomarkers useful to predict their effectiveness, still need to be fully elucidated. Increased understanding of AR blockade and its potential value in different BC subtypes and clinical testing of newer and more potent AR antagonists like darolutamide, is thus timely and necessary. The current study will enroll EBC female patients with differing BC subtypes, with the intent of characterizing the molecular alterations in BC tissue before and after a short-term exposure to darolutamide. Darolutamide is a new AR antagonist, that thus far has been found to be very well-tolerated. Studying the biological mechanisms in which darolutamide targets the AR in BC will be crucial in understanding its potential role in this disease, as well as providing the foundation for the rational development of darolutamide in the disease. Molecular profiling of tumor samples before and after darolutamide treatment may permit the identification of patients likely or unlikely to respond to the agent based on the biological and molecular characteristics of their tumors. Since it is essential to evaluate the molecular effects of darolutamide in tumors not exposed to chemotherapy or radiation, patients with untreated primary breast tumors amenable to a pre-treatment biopsy followed by a post-darolutamide sample at definitive surgery or prior to starting neoadjuvant systemic therapy (NAST) represent an ideal population to study. Although no direct benefit from the treatment with darolutamide is expected in the participating patients, the good tolerability and favourable safety profile of darolutamide at the selected dose, the proposed brief duration of treatment, and the fact that patients enrolled in the study will not be required to undergo any delay in definitive surgery/start of neoadjuvant therapy, should ensure the safety and ethical set-up of the trial.
Objectives	**Primary Objective:** ▪ To identify the molecular alterations that occur in human BC tissue, following short-term exposure to ODM-201 in female subjects with EBC.

	Secondary Objectives: ▪ To evaluate the safety and tolerability of short-term exposure to darolutamide in female subjects with EBC.
Study Design	This is a multi-center, open-label, tissue-acquisition study involving up to 60 patients with early-stage invasive BC. Patients with (a) suspicion of invasive BC based on clinical and/or radiological findings, or (b) a confirmation of invasive BC based on previous cytology/core/incisional biopsy will be invited to participate in the study: (a) In cases without pathological confirmation of BC diagnosis, signature of patient informed consent form (PICF) before BC confirmation biopsy is preferred to allow the collection of study-specific biopsies to be conducted. (b) For patients who have already had a confirmatory biopsy, they may also participate in the study if willing to undergo a subsequent study-specific biopsy procedure according to the protocol or if their diagnostic biopsy has been done according to the protocol requirements (in terms of number of cores/tissue, fresh frozen collection, etc). At time of PICF signature, patients will be registered in the study. Primary tumor samples will be collected by core needle biopsy or incisional biopsy (excisional biopsy will not be allowed). A minimum of three cores of tissue using a 14-gauge needle is recommended (or equivalent amount with an incisional biopsy). Two of the cores will be snap-frozen and the third one should be prepared in paraffin according to site's practice. In cases, it is not possible to provide enough material, collection of 2 cores (or equivalent amount with incisional biopsy) will be acceptable: one core will be snap-frozen and the other one will be prepared in paraffin. To be able to assess the molecular alterations after darolutamide exposure in different BC subtypes, patients being either triple-negative, or HR+/HER2 negative, or HER2 positive will be enrolled in the study (up to 20 patients with each of these subtypes, with an acceptable minimum of 8 evaluable patients in each cohort if enrollment of recommended 20 patients is not feasible for any reason). Eligible patients will be enrolled and receive darolutamide at a dose of 600 mg (2 × 300 mg tablets) b.i.d. to a daily dose of 1,200 mg. Protocol treatment should start within 5 days of enrollment, and within 42 days of the study-specific tumor sample collection. Minimum duration of treatment with darolutamide is 14 days with a recommended maximum duration of 21 days. It is recommended that the BC surgery/pre-NAST biopsy date is defined prior to starting protocol treatment. The start date of darolutamide (Day 1) will be derived as minus 14 to 21 days from the scheduled BC surgery/pre-NAST biopsy date. If for some reason BC surgery/pre-NAST biopsy takes place more than 21 days after treatment start (e.g. scheduling issues, delays, etc.), it is acceptable that patient receives darolutamide for more than 21 days and up to a maximum of 35 days. Patient should continue protocol treatment until the day prior to BC surgery/pre-NAST biopsy or "nil per os" (npo) is ordered. In these cases it is strongly recommended to have BC surgery/pre-NAST biopsy performed as soon as possible after 21 days of treatment are completed. Also, in any case that treatment is extended beyond 21 days, TRIO Medical Monitor should be contacted to discuss continuation of treatment and the need for assessments. At the time of BC surgery (if NAST is not indicated), or before NAST starts if indicated, BC tissue will be collected: two cores (or an equivalent amount of tissue), one fresh-frozen

	and one paraffin-embedded. End of Study (EoS) visit will occur 30 days (+/- 3 days) after the patient's last intake of darolutamide
Eligibility Criteria	**Inclusion Criteria** 1. Signed and dated PICF obtained prior to initiation of any study-specific procedure and treatment. 2. Female ≥ 18 years old. 3. Histologically proven invasive breast carcinoma (through either a core needle biopsy or an incisional biopsy) for which surgery is indicated as the primary treatment modality. Patients for which NAST is indicated are also eligible provided they are willing to undergo a biopsy after completing treatment with darolutamide and prior to NAST start. 4. Known ER, PgR and HER2 statuses. 5. Tumor must be confined to either the breast or to the breast and ipsilateral axilla (Note: patients with multifocal/multicentric *tumors are eligible).* Patient must have (according to TNM 7th edition rules): ▪ T1 with T ≥ 1.0 cm, T2 or T3 by at least one radiographic or clinical measurement ▪ Either clinically positive (N1 only) or clinically negative axillary nodes (N0) ▪ M0 6. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1. 7. Adequate organ function within 28 days prior to enrollment, as defined by the following criteria: ▪ Hematology: Hemoglobin ≥ 9.0 g/dL, Absolute neutrophil count (ANC) ≥ 1.5 × 109/L, Platelet count ≥ 100 × 109/L ▪ Liver function: Alanine aminotransferase (ALT) and aspartate transaminase (AST) ≤ 2.5 × upper limit of normal (ULN), Total bilirubin ≤ 1.5 × ULN (or ⍰ 3 times ULN for patients with documented Gilbert's syndrome or for whom indirect bilirubin concentrations suggest an extra-hepatic source of elevation). ▪ Renal function: Creatinine ≤ 2.0 × ULN 8. No more than 42 days should elapse from the day the study-specific tumor sample is taken at initial diagnosis (or subsequent procedure) to the day of the first intake of darolutamide. 9. Women of childbearing potential (WoCBP) must agree to use acceptable non-hormonal contraceptive methods of birth control from the day of the screening pregnancy test and up to 3 months after the last intake of darolutamide. 10. For WoCBP negative serum pregnancy test within 7 days of enrollment. 11. Willingness and ability to comply with scheduled visits, treatment plans, laboratory tests, and biopsies as detailed in the protocol. **Exclusion Criteria** 1. Any T0, Tis, T1 < 1.0 cm, T4; or N2-3; or M1 BC. 2. Bilateral invasive BC. 3. Patient that underwent excisional biopsy of the primary tumor. 4. Medical indication or patient desire to undergo BC surgery or start NAST prior to completing at least 14 days of treatment with darolutamide, and/or refusal of patient to undergo corresponding biopsy in case NAST is planned.

	5. Prior or concurrent systemic anticancer therapy for BC treatment (immunotherapy, biologic/targeted therapy, chemotherapy, investigational agents). 6. Prior or concurrent ipsilateral radiation therapy for invasive or noninvasive BC. 7. Prior or concurrent treatment or preventative use of any hormonal agent such as aromatase inhibitors (AI), fulvestrant, raloxifene, tamoxifen or other selective estrogen receptor modulators (SERM), or with any other hormonal agent used for the treatment or prevention of BC or for any other indication (e.g. osteoporosis). 8. Concurrent use of ovarian hormone replacement therapy. Prior treatment should be stopped at least 28 days prior to registration. 9. Prior or concurrent treatment with AR antagonists or CYP17 enzyme inhibitor. 10. Use of other investigational drug within 28 days of enrollment. 11. Major surgery within 28 days before enrollment 12. basal or squamous skin cancer, or carcinoma in situ of the cervix, or other noninvasive/in-situ neoplasm, all of which must have been adequately and radically treated. A patient with previous history of invasive malignancy (other than adequately and radically treated basal or squamous skin cancer) is eligible provided that she has been disease free for more than 5 years. 13. Severe or uncontrolled concurrent disease, infection or comorbidity. 14. Known active viral hepatitis, human immunodeficiency virus (HIV) or chronic liver disease. 15. Other serious illness or medical condition within 6 months before enrollment, including any of the following: Concurrent congestive heart failure New York Heart Association (NYHA) Class III or IV, severe/unstable angina pectoris, myocardial infarction, uncontrolled hypertension, coronary/peripheral artery bypass graft, high-risk uncontrolled arrhythmias, stroke. 16. Any contraindication to oral agents or gastrointestinal disorder or procedure which expects to interfere significantly with absorption of protocol treatment. 17. History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating Investigator. 18. Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial. 19. Known allergy to darolutamide or any of the excipients. 20. Pregnant or lactating patient
Protocol Treatment	Darolutamide will be given at a dose of 600 mg (2 x 300 mg tablets) b.i.d. to a daily dose of 1200 mg. The following timelines will be considered for darolutamide administration: ▪ Darolutamide shall be administered starting within 5 days of enrollment. ▪ No more than 42 days should elapse from the day the study-specific tumor sample is taken at initial diagnosis (or in a subsequent procedure) to the day of the first intake of darolutamide. ▪ Minimum duration of treatment will be 14 days prior to BC surgery or to the pre-NAST biopsy (if NAST is indicated). ▪ A maximum duration of 21 days of treatment is recommended. . It is recommended that the date of BC surgery/pre-NAST biopsy is defined prior to starting protocol treatment. The start date of darolutamide (Day 1) will be derived as minus 14 to 21 days from the scheduled BC surgery/pre-NAST biopsy date.

<table>
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Treatment will be given until the day prior to the BC surgery, or the day before pre-NAST biopsy or when npo is ordered, whichever occurs first.
If for some reason BC surgery/pre-NAST biopsy takes place more than 21 days after treatment start, it is acceptable that patient receives darolutamide for more than 21 days and up to a maxiumum of 35 days; patient should continue protocol treatment until the day prior to BC surgery/pre-NAST biopsy or npo is ordered. In these cases it is strongly recommended to have BC surgery/pre-NAST biopsy done as soon as possible after 21 days of treatment are completed. Also, in any case that treatment is extended beyond 21 days, TRIO Medical Monitor should be contacted to discuss continuation of treatment and the need for assessments

</td></tr>
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Up to 60 patients (a minimum of 8 evaluable patients and up to 20 patients in each breast cancer subtype: triple-negative, ER+/HER2 negative, HER2 positive) will be required for the performance of the molecular studies and possible hypothesis generation.
A patient will be considered evaluable if the following criteria are met:

Minimum duration of treatment with darolutamide is of 14 days, with a minimum of 10 consecutive days of treatment prior to the definitive surgery.
Mandatory tumor tissue is collected at screening and at surgery according to the protocol and submitted to the central lab.

</td></tr>
</table>

	▪ Adequacy for molecular assessment of the tumor tissue collected before and after the protocol treatment initiation. The adequacy will be evaluated by the central lab. Assessment of patient evaluability, safety and distribution of patients with respect to hormone receptor status and HER2 status will be performed by the Study Steering Committee (SSC) at given time points depending on the recruitment, primarily planned once 20, around 35 and once all patients have been enrolled and undergone the EoS per protocol. This will allow for the SSC to ascertain that the appropriate number of evaluable patients is being accrued, that more or less patients may be required, or in the extreme case, that accrual should close if the patient evaluability rate is very low and/or safety concerns arise. The SSC will define the evaluability of patients that discontinue treatment more than 3 days before surgery: molecular analyses may be taken into consideration for the decision about the evaulabilty of such patients. **Study Populations** ▪ Intention-to-treat (ITT) Population: the ITT population includes all patients who are enrolled in the study regardless of whether they have been treated or not. ▪ Safety Population: the Safety population includes all subjects who have taken at least one tablet of darolutamide. ▪ Evaluable population: includes all patients who are considered evaluable per protocol.

5.2. Protocol Synopsis – Protocol v5.0 (Germany)

Protocol Title	A Presurgical Tissue-Acquisition Study to Evaluate Molecular Alterations in Human Breast Cancer Tissue Following Short-Term Exposure to the Androgen Receptor Antagonist Darolutamide (ODM-201)
Protocol #	TRIO030
Study Duration	Enrollment is expected to run over a 12-month period approximately. The overall study is expected to last around 14 months (from First Patient In until Last Patient Last Visit).
Sponsor/Study Chair	This is a TRIO-sponsored study which will be chaired by Dr. Dennis Slamon (Professor of Medicine, Chief, Division of Hematology-Oncology, UCLA's Department of Medicine, CA, USA). The study is financially supported by Bayer.
Participating Investigators/Sites	Approximately 20 sites in North America and Europe will participate in the study.
Target Population	Women with early-stage breast cancer (BC) candidates for surgery as primary treatment modality or candidates for neoadjuvant treatment who accept to undergo a second biopsy before starting such therapy
Background and Rationale	Despite progresses made in diagnostic techniques and treatments for BC, there is still a significant number of patients undergoing treatment for early BC (EBC) who will relapse or patients with advanced BC that do not respond or progress to their treatments. The medical need for new and potentially more active and better tolerated agents in BC remains very high. Endocrine treatment is one of the strategies with a major therapeutic value in patients with estrogen-receptor (ER) positive tumors. Although, the role of ER and progesterone receptor (PgR) is well established in BC as predictive and prognostic factors, little is known about the clinical significance of the androgen receptor (AR). Preclinical studies show that the androgen signaling pathway plays a critical role in the development of normal and malignant breast tissue. AR is expressed in approximately 80% and 60% of primary and metastatic BC, respectively. Its expression varies across the different subtypes, approximately 84-95% in ER+ tumors, 50-63% in ER-/HER2+ cases and 10-53% in TNBC. In ER+ BC it has been suggested that acquired resistance to antiestrogen therapies could result from adaptation from estrogen dependence to androgen dependence. Recently, clinical

	reports of AR antagonism have been promising, especially in TNBC. Bicalutamide and enzalutamide have been tested in phase II trials in TNBC and have shown promising clinical activity. These developments in combination with an improved understanding of AR signaling in different BC subtypes led to the renewed interest in targeting AR. However, the value of AR antagonists in the management of BC, the molecular alterations that occur after exposure to these agents and whether there are biomarkers useful to predict their effectiveness, still need to be fully elucidated. Increased understanding of AR blockade and its potential value in different BC subtypes and clinical testing of newer and more potent AR antagonists like darolutamide, is thus timely and necessary. The current study will enroll EBC female patients with differing BC subtypes, with the intent of characterizing the molecular alterations in BC tissue before and after a short-term exposure to darolutamide. Darolutamide is a new AR antagonist, that thus far has been found to be very well-tolerated. Studying the biological mechanisms in which darolutamide targets the AR in BC will be crucial in understanding its potential role in this disease, as well as providing the foundation for the rational development of darolutamide in the disease. Molecular profiling of tumor samples before and after darolutamide treatment may permit the identification of patients likely or unlikely to respond to the agent based on the biological and molecular characteristics of their tumors. Since it is essential to evaluate the molecular effects of darolutamide in tumors not exposed to chemotherapy or radiation, patients with untreated primary breast tumors amenable to pre-treatment biopsy followed by a post-darolutamide sample at definitive surgery or prior to starting neoadjuvant systemic therapy (NAST) represent an ideal population to study. Although no direct benefit from the treatment with darolutamide is expected in the participating patients, the good tolerability and favourable safety profile of darolutamide at the selected dose, the proposed brief duration of treatment, and the fact that patients enrolled in the study will not be required to undergo any delay in definitive surgery/start of neoadjuvant therapy, should ensure the safety and ethical set-up of the trial
Objectives	**Primary Objective:** ▪ To identify the molecular alterations that occur in human BC tissue, following short-term exposure to darolutamide in female patients with EBC. **Secondary Objectives:** ▪ To evaluate the safety and tolerability of short-term exposure to darolutamide in female patients with EBC.
Study Design	This is a multi-center, open-label, tissue-acquisition study involving up to 60 patients with early-stage invasive BC. Patients with (a) suspicion of invasive BC based on clinical and/or radiological findings, or (b) a confirmation of invasive BC based on previous cytology/core/incisional biopsy will be invited to participate in the study: (a) In cases without pathological confirmation of BC diagnosis, signature of patient informed consent form (PICF) before BC confirmation biopsy is preferred to allow the collection of study-specific biopsies to be conducted. (b) For patients who have already had a confirmatory biopsy, they may also participate in the study if willing to undergo a subsequent study-specific biopsy procedure according to the protocol or if their diagnostic biopsy has been done according to the protocol requirements (in terms of number of cores/tissue, fresh frozen collection, etc). At time of PICF signature, patients will be registered in the study. Primary tumor samples will be collected by core needle biopsy or incisional biopsy (excisional biopsy will not be allowed). A minimum of three cores of tissue using a 14-gauge needle is recommended (or equivalent

	amount with an incisional biopsy). Two of the cores will be snap-frozen and the third one should be prepared in paraffin according to site's practice. In cases, it is not possible to provide enough material, collection of 2 cores (or equivalent amount with incisional biopsy) will be acceptable: one core will be snap-frozen and the other one will be prepared in paraffin. To be able to assess the molecular alterations after darolutamide exposure in different BC subtypes, patients being either triple-negative, or HR+/HER2 negative, or HER2 positive will be enrolled in the study (up to 20 patients with each of these subtypes, with an acceptable minimum of 8 evaluable patients in each cohort if enrollment of recommended 20 patients is not feasible for any reason). Eligible patients will be enrolled and receive darolutamide at a dose of 600 mg (2 × 300 mg tablets) b.i.d. to a daily dose of 1,200 mg. Protocol treatment should start within 7 days of confirmation of BC diagnosis (i.e. availability of pathological report). Minimum and expected treatment duration should be 14 days and ODM-201 should continue until the day prior to BC surgery/pre-NAST biopsy or when the subject is ordered to stop all oral intake, whichever occurs first. In any case, surgery must be planned within 21 days of confirmation of BC diagnosis. At the time of surgery (if NAST is not indicated), or before NAST starts if indicated, BC tissue will be collected: two cores (or an equivalent amount of tissue), one fresh-frozen and one paraffin-embedded. End of Study (EoS) visit will occur 30 days (+/- 3 days) after the patient's last intake of darolutamide. *Note: If the BC surgery/pre-NAST biopsy that is initially planned to take place within 21 days after BC diagnosis is delayed due to unforeseen circumstances (e.g. scheduling issues, delays, etc.) it is acceptable that the subject continues receiving ODM-201 up to a maximum of 35 days. However, in this case Investigator should contact the TRIO Medical Monitor to discuss the continuation of treatment and the need for assessments.*
Eligibility Criteria	**Inclusion Criteria** 1. Signed and dated PICF obtained prior to initiation of any study-specific procedure and treatment. 2. Female ≥ 18 years old. 3. Histologically proven invasive breast carcinoma (through either a core needle biopsy or an incisional biopsy) for which surgery is indicated as the primary treatment modality. Patients for which NAST is indicated are also eligible provided they are willing to undergo a biopsy after completing treatment with darolutamide and prior to NAST start. 4. Known ER, PgR and HER2 statuses. 5. Tumor must be confined to either the breast or to the breast and ipsilateral axilla (Note: patients with multifocal/multicentric tumors are eligible). Patient must have (according to TNM 7th edition rules): ▪ T1 with T ≥ 1.0 cm, T2 or T3 by at least one radiographic or clinical measurement ▪ Either clinically positive (N1 only) or clinically negative axillary nodes (N0) ▪ M0 6. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1. 7. Adequate organ function within 28 days prior to enrollment, as defined by the following criteria: ▪ Hematology: Hemoglobin ≥ 9.0 g/dL, Absolute neutrophil count (ANC) ≥ 1.5 × 109/L, Platelet count ≥ 100 × 109/L ▪ Liver function: Alanine aminotransferase (ALT) and aspartate transaminase (AST) ≤ 2.5 × upper limit of normal (ULN), Total bilirubin ≤ 1.5 × ULN (or ⍰ 3 times ULN for patients with documented Gilbert's syndrome or for whom indirect bilirubin concentrations suggest an extra-hepatic source of elevation). ▪ Renal function: Creatinine ≤ 2.0 × ULN 8. No more than 7 days should elapse from the confirmation of BC diagnosis (i.e. availability

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Signature Manifest

Document Number: TRIO030-DM-0025 **Revision:** 03

Title: Statistical Analysis Plan

All dates and times are in Mountain Time.

TRIO030 SAP & Mock Shells

Approval

Name/Signature	Title	Date	Meaning/Reason
Yan Yang (YYANG)		24 Sep 2019, 02:37:36 PM	Approved
Tamara Irvin (TIRVIN)		24 Sep 2019, 02:58:11 PM	Approved
Pablo Millan (PMILLAN)		25 Sep 2019, 08:28:26 AM	Approved
Ana Popovic (APOPOVIC)		25 Sep 2019, 10:31:26 AM	Approved
Melissa Burton (MJOHNSON)		26 Sep 2019, 09:45:06 AM	Approved
Brandy Thibodeau (BTHIBODE)		26 Sep 2019, 10:17:54 AM	Approved
Stephanie Carrez (SCARREZ)		27 Sep 2019, 02:28:30 AM	Approved
Karen Afenjar (KAFENJAR)		30 Sep 2019, 07:53:27 AM	Approved

Set Effective Date

Name/Signature	Title	Date	Meaning/Reason
Brandy Thibodeau (BTHIBODE)		30 Sep 2019, 08:01:30 AM	Approved